

REVIEW

Follicular helper T cells and follicular regulatory T cells in the immunopathology of primary Sjögren's syndrome

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Abstract

Primary Sjögren's syndrome (pSS) is a chronic autoimmune disease, characterized by lymphocytic infiltration into exocrine glands, which causes dry eyes, dry mouth, and systemic damage. Although the precise etiology of pSS is not clear yet, highly activated B cells, abundant anti-SSA/Ro, and anti-SSB/La autoantibodies are the hallmarks of this disease. Follicular helper T cells (Tfh), a subset of CD4⁺T cells, with cell surface receptors PD-1 and CXCR5, express ICOS, transcription factor Bcl-6, and a cytokine IL-21. These cells help in the differentiation of B cells into plasma cells and stimulate the formation of germinal center (GC). Previous studies have demonstrated abundant Tfh cells in the peripheral blood and salivary glands (SGs) of the patients with pSS, correlated with extensive lymphocytic infiltration of the SGs and high disease activity scores. Patients with pSS who are treated with abatacept (CTLA-4 Ig) show fewer circulating Tfh cells, reduced expression of ICOS, and lower disease activity scores. Recently identified follicular regulatory T (Tfr) cells, a subset of regulatory T cells, control the function of Tfh cells and the GC reactions. Here, we summarize the observed alterations in Tfh and Tfr cell numbers, activation state, and circulating subset distribution in pSS. Our goal is to improve the understanding of the roles of Tfh and Tfr cells (surface marker expression, cytokine production, and transcription factors) in the pathogenesis of pSS, thus contributing to the identification of candidate therapeutic agents for this disease.

KEYWORDS

primary Sjögren's syndrome, Tfh cells, Tfr cells, CXCR5, PD-1, germinal center

1 | INTRODUCTION

Primary Sjögren's syndrome (pSS) is a classic autoimmune disease characterized by xerostomia and xerophthalmia.¹ Extra-glandular manifestations (EGMs) are very common in patients, which consist of interstitial pneumonia, autoimmune cytopenia, polyarthritis, Raynaud's phenomenon, lymphadenopathy, vasculitis, renal disease, polyneuropathy, and myositis, and severe cases can be life threatening. This disease affects 0.01–0.72% of the global population.² pSS is a major chronic disease, affecting mainly middle-aged and elderly women.³ Moreover, patients with pSS have a 6.5-fold higher risk of

developing non-Hodgkin's lymphoma, which is the most severe of the known autoimmune diseases.^{4,5} The pathologic features of pSS include chronic autoimmune sialadenitis, in which T and B lymphocytes are abnormally activated. In addition, plasma cells, which are differentiated from B cells, continuously produce autoantibodies, leading to immune damage.^{5–8} However, the detailed pathogenesis is still not clear. Current therapies for patients with pSS are still unsatisfied.^{9,10} Lack of specific therapeutic drugs makes in-depth research on the pathogenesis of pSS particularly important.⁹ Furthermore, a better understanding of the mechanisms of interaction between T and B lymphocytes is expected to yield novel targets for the treatment of this disease.

It is well known that hyperglobulinemia is an important clinical feature of this autoimmune disease. B cell hyperactivity is manifested by a higher titer of anti-SSA/Ro and anti-SSB/La antibodies, IgG, and rheumatoid factor. The highly activated T and B cells both infiltrate the salivary gland (SG). The early-infiltrating cells in the SGs are mainly

Abbreviations: Ascl2, Achaete-Scute Homologue 2; cTfh, circulating Tfh; DCs, dendritic cells; EGMs, extra-glandular manifestations; ESSDAI, European League Against Rheumatism Sjögren's syndrome disease activity index; GC, germinal center; MSCs, mesenchymal stem cells; mTOR, mammalian target of rapamycin; pSS, primary Sjögren's syndrome; RTX, Rituximab; SGs, salivary glands; Tfh, follicular helper T cells; Tfr, follicular regulatory T cells; Tregs, regulatory T cells; tTfh, tissue Tfh.

CD4⁺T cells, whereas B lymphocytes appear later in the glandular lesion. There is crosstalk between T and B cells, leading to continuous inflammation, as a result of the loss of the glandular architecture and impaired function of the SG. Different CD4⁺T cell subsets may be involved in pSS-related autoimmunity. The normal balance between different subsets of CD4⁺T cells, that is, Th1, Th2, Th17, follicular helper T cells (Tfh), and regulatory T cells (Tregs), is disturbed in patients with pSS.^{11,12} Th1 and Th17 cells seem to infiltrate the glands at an early disease stage, as evidenced by the detection of IFN- γ and IL-17, whereas infiltration of Tfh cells occurs at later stages.¹³ The synchronous time course of Tfh and B cells both appearing late in the SG implies an interaction between Tfh and B cells in the development of pSS.

Ectopic germinal center (GC) formation in the glandular tissue is another feature of pSS.¹⁴ GC is also found in the mouse model of pSS.¹⁵ The formation of ectopic GC is preceded by aggregation of T and B cells in inflammatory sites in pSS. The number of GC in SGs is associated with the severity of inflammation, and enhanced anti-SSA, anti-SSB autoantibody production. Moreover, the formation of ectopic GC is associated with a higher risk of developing B cell lymphoma.¹⁴ In GC, Ag-specific B cells undergo proliferation, isotype switching, and somatic hypermutation, where high-affinity GC B cells are selected and eventually differentiate into either memory cells or long-lived plasma cells, producing an affinity maturation of serum Antibody. Tfh cells in GC can promote B cell differentiation, GC formation, and antibody production, and also are associated with the development of pSS disease. Follicular regulatory T (Tfr) cells, described by 3 groups in 2011, demonstrated characters of both Tfh cells and Tregs, thereby regulating the GC response and antibody affinity.^{16–18} Here, we will summarize the latest data on the expression, distribution, and functions of Tfh and Tfr cells in patients with and mouse models of pSS, as well as analyze potential therapeutic targets. We also discuss future research prospects for further understanding this disease.

2 | DESCRIPTION OF Tfh CELLS IN HUMAN AND MICE

In human tonsils, CD4⁺T cells expressing CXCR5, which is required for their migration into B-cell follicles in secondary lymphoid organs, have a better capacity to induce immunoglobulin production in B cells in vitro, compared with CD4⁺T cells lacking CXCR5 expression. Based on their localization and functions, tonsillar CXCR5⁺CD4⁺T cells are defined as Tfh cells.^{19–21} A similar subset of CD4⁺T cells is also found in mouse lymph nodes.²² Profiling of cytokine production and gene expression in human and mouse Tfh cells showed that these cells are distinct from Th1, Th2, and Th17 cells, which can help differentiation and isotype switching of B cells mainly in their ability to use IL-21 and CD40L^{23–25} for signaling. In addition to CXCR5, ICOS and the inhibitory receptor PD-1 are the other 2 important makers for Tfh cells. ICOS is essential for the generation of Tfh cells and GC responses in humans and mice.²⁶ The GC is crucial for the formation of long-lived, high-affinity T cell-dependent B cell responses. Patients with

ICOS deficiency suffer from common variable immunodeficiency and have significantly impaired GC formation in lymphoid tissues, drastically decreased circulating memory Tfh cells in the blood, and a severe deficiency of memory B cells.²⁷ Tfh cells in human GCs are currently defined by their high expression of CXCR5, ICOS, and PD-1.²⁸ However, ICOS is not a useful marker for defining Tfh cells in the GC of mice, since ICOS expression is similar in GC Tfh cells and Tfh precursors in mice.

When the transcription factor Bcl-6 is identified as essential for Tfh cell generation in humans and mice,^{29–31} these cells are recognized as an independent subset of Th, distinct from Th1, Th2, and Th17 cells. IL-21 influences the expression of *BCL6*, *BLIMP-1*, and *MAF* genes, all of which encode transcription factors central to Tfh cell development.³² Although Bcl-6 is essential for Tfh cells, it is not a reliable biomarker for mouse Tfh cells in lymph tissue, as its expression decreases in these cells after antigen exposure.³³ Besides Bcl-6, Achaete-Scute Homologue 2 (*Ascl2*) is another transcription factor that is selectively up-regulated in human and mouse Tfh cells.^{34,35} *Ascl2* promotes T cell migration into B-cell follicles and aids the development of Tfh cells in mice. Deletion of *Ascl2* impairs the Tfh cell development and the GC response in mice.³⁴

The development of Tfh cells differs between mice and humans. In humans, the cytokine TGF- β interacts with cofactors IL-12 and IL-23 to promote the expression of multiple Tfh molecules, including CXCR5, IL-21, and Bcl-6, on the activated naïve CD4⁺T cells.³⁶ Unlike in human CD4⁺T cells, TGF- β suppresses the differentiation of Tfh cells from CD4⁺T cells in mice by decreasing the expression of Tfh-specific molecules IL-21, ICOS, and Bcl-6.³⁶ STAT3 is required for Tfh cell generation in humans, and the people carrying mutations in STAT3 have altered antibody responses, and reduced memory Tfh cells in circulation. STAT3 is the main transmitter of IL-21 and IL-6 signals in T and B cells. Since the B cell responses to IL-10 and IL-21 are severely altered in STAT3-deficient subjects, the number of circulating memory Tfh cells is reduced in them. STAT3 can bind to the *Bcl-6* gene locus and promote its expression and strongly induce *Blimp1* expression in B cells and T cells. Considering that these 2 transcription factors (*Bcl-6* and *Blimp1*) are directly antagonistic to each other, the balance between them ultimately determines the final differentiation fate of Tfh cells.

3 | MEMORY Tfh SUBSETS IN HUMAN BLOOD

Tfh cells located in GC are essential for their formation, where important processes like B cell clonal expansion and development of memory B cell and long-lived plasma cells occur.³⁷ Circulating Tfh (cTfh) in human peripheral blood also can support B cell differentiation and class switching.³⁸ Recent studies indicate that circulating CXCR5⁺CD4⁺T cells contain long-lived memory cells that share functional properties with Tfh cells,³⁹ and hence, these are termed as circulating memory Tfh cells. Unlike GC Tfh cells, circulating memory Tfh cells do not express the Bcl-6 protein,^{40,41} indicating that Bcl-6 is dispensable for the maintenance of cTfh cells. Heterogeneous

subsets of memory Tfh in the human blood include several populations with unique phenotypes and functions, depending on the presence of the chemokine receptors CXCR3, CCR6, and CCR7, immunoregulatory molecule PD-1, and costimulatory molecule ICOS.³⁹

Based on the expression patterns of CXCR3 and CCR6, 3 major subsets can be defined: (1) CXCR3⁺CCR6⁻ cells that share properties with Th1 cells (circulating memory Tfh1 cells), (2) CXCR3⁻CCR6⁻ cells resembling Th2 cells (circulating memory Tfh2 cells), and (3) CXCR3⁻CCR6⁺ cells resembling Th17 cells (circulating memory Tfh17 cells). Circulating memory Tfh2 and Tfh17 cells can induce naive B cells to produce immunoglobulins and to switch isotypes through secretion of IL-21. Furthermore, circulating memory Tfh2 cells promote the secretion of IgG and IgE, whereas circulating memory Tfh17 cells induce the secretion of IgG and IgA.⁴² Thus, cTfh2 and cTfh17 cells are efficient B cell helper cells with a distinct capacity to regulate immunoglobulin isotype switching. In contrast, circulating memory Tfh1 cells cannot induce naive B cells to produce immunoglobulins.^{38,43} However, when Tfh1 cells become activated by ICOS⁺, they induce the differentiation of memory B cells into plasma cells by secreting IL-21 and IL-10.⁴³

Based on the expression levels of ICOS, PD-1, and CCR7, 3 other subsets of Tfh cells can be defined: 1 activated subset (CCR7^{lo}ICOS⁺PD-1^{hi}) and 2 quiescent subsets (CCR7^{int}ICOS⁻PD-1⁺ and CCR7^{hi}ICOS⁻PD-1⁻). In contrast to Tfh cells in secondary lymphoid organs, ICOS expression is limited to a small population of circulating Tfh cells in healthy subjects, suggesting that the majority of cTfh cells from healthy individuals are at a quiescent state; this population substantially increases after vaccination, defining the activated memory Tfh cells.³⁹ The expression of CCR7 in circulating memory Tfh cells negatively correlates with that of PD-1. The activated CCR7^{lo}ICOS⁺PD-1^{hi} populations in circulating memory Tfh2 and Tfh17 cells may represent the most efficient helpers.³⁹ Circulating CCR7^{lo}CXCR5⁺PD-1^{hi}CD4⁺T precursor cells can rapidly differentiate into Tfh cells and promote antibody production.⁴⁰ The proportion of circulating CCR7^{lo}CXCR5⁺PD-1^{hi}CD4⁺T precursor cells correlates well with Tfh cell activity, including autoantibody production.^{40,44} However, it remains to be seen whether cTfh cells are the precursors of GC Tfh cells, or are derived from them.⁴²

4 | Tfh CELLS IN PATIENTS WITH PSS AND ANIMAL MODEL

pSS is characterized by the presence of high titers of autoantibodies against SSA/Ro and SSB/La. The GC in the SG can be detected by light microscopy within the otherwise normal epithelium and consists of a specialized subset of Tfh cells, B cells, and dendritic cells (DCs). During GC reactions, Tfh cells emerge as a distinct subset of CD4⁺T helper cells that promote the development and activation of B cells.^{45,46} Autoreactive B cells in pSS are derived from GC reactions. Therefore, the role of Tfh cells in the pathogenesis of human pSS is crucially important. Difficulties in investigating Tfh cells from human secondary lymphoid organ and the easy accessibility of test circulating

Tfh-like cells (cTfh) in human blood and tissue Tfh (tTfh) cells in glandular tissue provides an ideal opportunity to study these cells in pSS.

Increased levels of cTfh cells in the blood^{41,47-56} and tTfh cells in SGs^{49,57-61,64} have been reported in patients with pSS. The phenotype of cTfh cells is shown in Table 1. The main surface markers of cTfh cells are ICOS, CXCR5, PD-1, and CD4, with IL-21 being the crucial cytokine of cTfh cells. In 2010, cTfh cells were first defined as ICOS^{hi}CXCR5⁺CD4⁺T cells or PD-1^{hi}CD4⁺T cells isolated from the blood from a small sample of 17 patients with pSS. Both cell types were expressed at high levels in pSS patients compared with the healthy controls, but no investigation of the association between cTfh cells and pSS disease activities was performed.⁴¹ cTfh cells reflected GC Tfh function, which promotes the high-affinity autoantibodies in lupus disease, but this is not clear in pSS. Bcl-6 expression is lower in cTfh cells than in tonsillar Tfh cells in healthy individuals, but this has not been tested in patients with pSS.⁴¹ In 2012 and 2014, circulating CXCR5⁺CD4⁺T cells were identified as cTfh cells in 25 and 24 pSS patients.^{47,49} Interestingly, 1 subtype of cTfh cells, CXCR3⁻CCR6⁺CXCR5⁺CD4⁺T cells (cTfh17) express PD-1, ICOS, CD40L, IL-21, Bcl-6 (mRNA data), and lower CCR7, but other 2 subtypes such as cTfh1 and cTfh2 cells express less PD-1, ICOS, CD40L, IL-21, no Bcl-6, higher CCR7. cTfh17 cells are more abundant in patients with pSS, positively correlating with disease activity, total IgG, anti-SSA, and anti-SSB autoantibody titers.⁴⁷ However, another study has shown contrasting results indicating that CXCR5⁺CD4⁺cTfh cells do not correlate with the titer of ANA and IgG levels. This is the main reason that CXCR5⁺CD4⁺cTfh cells are in a quiescent state.⁴⁹ Two other reports define ICOS⁺CXCR5⁺PD-1⁺CD4⁺T cells as cTfh cells.^{48,51} ICOS is an active biomarker for cTfh cells, and cTfh cells from patients with pSS show a 3-fold higher expression of ICOS compared with the healthy controls.⁵² Upon activation, ICOS is highly up-regulated, together with CXCR5 and PD-1, turning Tfh cells into a hyperactive state in patients with pSS. Another study also shows that these activated ICOS⁺CXCR5⁺PD-1⁺CD4⁺T cells are increased in pSS patients, and are associated with disease activity.⁵⁶ cTfh cells are significantly higher in pSS patients with EGMs that demonstrate higher IL-12 or IL-21 levels. cTfh cells are positively related to total IgG, anti-SSA, anti-SSB antibody levels, and glandular inflammation.^{48,51} This suggests that activated ICOS⁺cTfh cells are closely related to systemic clinical symptoms. Such alterations in circulating memory Tfh cells, especially cTfh17 cells and activated ICOS⁺cTfh cells, might reflect an overall increase in the efficient helper cells that promote the generation of antibodies in lymphoid organs and/or inflammatory sites in patients with pSS.

Interestingly, IL-21 producing-cTfh cells correlate with self-reactive B cells (CD19⁺CD38^{hi}CD24^{hi}CD27⁻ transitional B cells and mature-naive B cells) in pSS patients with EGMs.⁵¹ When B cell tolerance breaks, along with the hyperactivation of Tfh cells, self-reactive B cells accumulate in the peripheral blood and contribute to the autoimmunity in pSS. cTfh cells are related to disease activity and pSS specific anti-SSA and anti-SSB antibodies,^{47,48,50,51} suggesting that cTfh cells promote the development of this disease by helping B cells with the production of higher levels of anti-SSA and anti-SSB antibodies. This

TABLE 1 T follicular helper cells (Tfh) and follicular regulatory T cells (Tfr) in patients with primary Sjögren's syndrome

Study	Patients	Phenotype	Functional marker	Association with disease parameters
cTfh ⁴¹	17 pSS patients	ICOS ^{hi} CXCR5 ⁺ CD4 ⁺ T cells (cTfh)↑, PD-1 ^{hi} CD4 ⁺ T cells (cTfh)↑	NA	NA
cTfh ⁴⁷	25 pSS patients	CXCR5 ⁺ CD4 ⁺ T cells (cTfh)↑, CCR6 ⁺ CXCR5 ⁺ CD4 ⁺ T cells (cTfh17)↑	cTfh17 express PD-1, ICOS, CD40L, and IL-21, Bcl-6, lower CCR7	cTfh17 cells positively correlated with the level of total IgG, anti-SSA, anti-SSB antibodies, and ESSDAI score.
cTfh ⁴⁸	55 pSS patients	ICOS ⁺ CXCR5 ⁺ PD-1 ⁺ CD4 ⁺ T cells (cTfh)↑	cTfh expressed ICOS	cTfh cells were higher in pSS with EGMs, higher IL-12, or higher IL-21 levels. cTfh cells were positively related to total IgG, anti-SSA, -SSB antibody levels, and gland inflammation.
cTfh, tTfh ⁴⁹	24 pSS patients	CXCR5 ⁺ CD4 ⁺ T cells (cTfh, tTfh)↑	NA	cTfh and tTfh cells both are increased in patients with pSS. tTfh cells positively correlated with blood CD19 ⁺ CD27 ⁺ memory B cells and CD19 ⁺ CD27 ^{hi} plasma cells. cTfh cells decreased after treatment.
cTfh ⁵⁰	48 pSS patients	CXCR5 ⁺ PD-1 ⁺ CD4 ⁺ T cells (cTfh)↑	NA	cTfh cells were positively correlated with serum anti-La/SSB levels and the ESSDAI score.
cTfh ⁵¹	25 pSS patients	ICOS ⁺ CXCR5 ⁺ PD-1 ⁺ CD4 ⁺ T cells (cTfh)↑, IL-21 ⁺ CXCR5 ⁺ PD-1 ⁺ CD4 ⁺ T cells (IL-21 ⁺ cTfh)↑	cTfh expressed IL-21	Higher cTfh and IL-21 ⁺ cTfh cells in pSS patients with EGMs, anti-Ro/SSA antibody-positive group.
cTfh ⁵²	15 pSS patients	CD45RA ⁻ CXCR5 ⁺ PD-1 ⁺ CD4 ⁺ T cells (cTfh)↑, CD45RA ⁻ ICOS ⁺ CXCR5 ⁺ PD-1 ⁺ CD4 ⁺ T cells (cTfh)↑	cTfh expressed ICOS	Abatacept reduced cTfh cells, serum IL-21, CXCL13, plasmablasts, total IgG, anti-SSA, -SSB antibody titers, and ICOS expression in parotid gland tissue. Reduced ICOS expression on cTfh cells correlated significantly with lower ESSDAI scores during treatment.
cTfh ⁵³	24 pSS patients	CD45RA ⁻ FoxP3 ⁻ CXCR5 ⁺ PD-1 ⁺ CD4 ⁺ T cells (cTfh)↑	cTfh expressed IL-21	Rituximab therapy reduced cTfh cells, IL-21 ⁺ CD4 ⁺ T cells, and serum IL-21. The decrease in circulating Tfh cells was associated with lower disease activity and lower autoantibody titers after treatment.
cTfh, cTfr ⁵⁵	18 pSS patients	CD25 ⁻ CD127 ^{hi} CXCR5 ⁺ CD4 ⁺ T cells (Tfh) did not increase, CCR7 ^{lo} PD-1 ^{hi} cTfh↑, CD25 ⁺ CD127 ^{lo} CXCR5 ⁺ CD4 ⁺ T cells (cTfr)↑	NA	CCR7 ^{lo} PD-1 ^{hi} cTfh cells are increased in pSS patients with high degree of focal lymphocytic sialadenitis. CCR7 ^{lo} PD-1 ^{hi} cTfh cells are correlated with disease activity scores and plasma cells.
tTfh ⁵⁷	8 pSS patients	IL-21 ⁺ CXCR5 ⁺ T cells (tTfh)	tTfh expressed IL-21	The more the lymphocyte infiltrated, the more IL-21 expression in SGs.
tTfh ⁵⁸	54 pSS patients	Bcl-6 ⁺ CXCR5 ⁺ T cells (tTfh)	tTfh expressed IL-21.	The presence of tTfh cells was identified in the SGs of pSS patients. Tfh-related molecules Bcl-6, CXCR5, IL-21 were detected in the GC ⁺ SGs by immunohistochemic staining. Bcl-6 mRNA was higher in SGs with GC ⁺ of pSS patients. tTfh was associated with strong lymphocytic infiltration.
tTfh ⁵⁹	10 pSS patients	Bcl-6 ⁺ CD3 ⁺ T cells (tTfh)↑	tTfh expressed CD84, PD-1, Bcl-6	tTfh was close to Bcl-6 ⁺ B cells in the typical formation of GC. Presence of tTfh may predict disease development.
tTfh ⁶¹	29 pSS patients	NA	NA	tTfh cells were increased in SGs of pSS patients. tTfh cells correlated with the expression of CXCL13, lymphocytic focus scores, local B cell hyperactivity, and anti-SSA positivity.
cTfh ⁶³	15 pSS patients	CCR9 ⁺ CD4 ⁺ T cells (cTfh)↑	NA	cTfh were increased in patients with pSS.

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TABLE 1 (Continued)

Study	Patients	Phenotype	Functional marker	Association with disease parameters
cTfh, tTfh ⁶⁴	26 pSS patients	CCR9 ⁺ CD4 ⁺ T cells (cTfh, tTfh)↑	cTfh expressed ICOS, PD-1, IL-21, IL-17, and, IFN- γ , IL-7 α . tTfh expressed ICOS	cTfh promoted the IgG production of B cells. No significant correlations of tTfh cells with the lymphocytic focus score, percentage of IgG ⁺ and IgM ⁺ plasma cells, or serum IgG levels.
cTfh, tTfh, cTfr, tTfr ^{60, 84}	16 pSS patients	CD45RO ⁺ CD25 ⁻ Foxp3 ⁻ CXCR5 ⁺ CD4 ⁺ T cells (cTfh) do not increase, ICOS ⁺ PD-1 ⁺ T cells (tTfh)↑, Foxp3 ⁺ CXCR5 ⁺ CD4 ⁺ T cells (cTfr)↑ Foxp3 ⁺ CXCR5 ⁺ T cells (tTfr)↑	cTfh expressed ICOS and PD-1	pSS patients had normal cTfh cells, ICOS ⁺ PD-1 ⁺ cTfh cells, cTfh1, cTfh2, and cTfh17. However, activated ICOS ⁺ PD-1 ⁺ cTfh cells were associated with disease activity and anti-SSA antibody. cTfr/cTfh ratio was increased, associating with ectopic lymphoid neogenesis, lymphocyte infiltration in salivary gland of pSS patients. cTfr/cTfh ratio was recommended as a marker for pSS diagnosis.
cTfh, cTfr ⁵⁶	44 pSS patients	CD45RA ⁻ Foxp3 ⁻ CXCR5 ⁺ CD4 ⁺ T cells (cTfh)↑, ICOS ⁺ PD-1 ⁺ cTfh↑ Foxp3 ⁺ CD4 ⁺ CXCR5 ⁺ T cells (cTfr)↑	cTfh expressed ICOS and PD-1	Activated ICOS ⁺ PD-1 ⁺ cTfh cells were increased in patients with pSS, and associated with disease activity. cTfr cells and cTfr/cTfh ratio were increased in pSS patients, but not associated with gland inflammation.

Tfh, follicular helper T cells; Tfr, follicular regulatory T cells; cTfh, circulating Tfh; pSS, primary sjögren's syndrome; NA, not applicable; ESSDAI, European League Against Rheumatism Sjögren's syndrome disease activity index; EGMs, extra-glandular manifestations; tTfh, tissue Tfh; cTfr, circulating Tfr; SG, salivary gland; GC, germinal center

TABLE 2 T follicular helper cells (Tfh) and follicular regulatory T cells (Tfr) in Sjögren's syndrome animal model

Study	Animal	Phenotype	Functional marker	Association with disease parameters
Splenic Tfh, Tfr ³⁵	NFS/sld mice that underwent thymectomy surgery	Foxp3 ⁻ CD62L ⁻ CXCR5 ⁺ PD-1 ⁺ CD4 ⁺ cells (Tfh)↑, Foxp3 ⁺ CD62L ⁻ CXCR5 ⁺ PD-1 ⁺ CD4 ⁺ cells (Tfr)	Tfh cells expressed Ascl2	Splenic Tfh cells were positively correlated with the severity of submandibular glands lesions.
Splenic Tfh ⁷⁸	6 NOD/ShiLtJ mice	CXCR5 ⁺ CD4 ⁺ T cells (Tfh)↑	NA	MSCs treatment ameliorates sialadenitis in the mouse model partly through reducing Tfh cells.
Lymphatic Tfh, Tfr ⁸¹	C57BL/6 background mice immunized with SG proteins	Foxp3 ⁻ CXCR5 ⁺ PD-1 ⁺ CD4 ⁺ T cells (Tfh), Foxp3 ⁺ CXCR5 ⁺ PD-1 ⁺ CD4 ⁺ T cells (Tfr)	NA	Tfh cells and Tfr cells were found in cervical lymph nodes of Sjögren syndrome mouse model.
Splenic Tfh, Tfr ⁸⁶	9 NOD/ShiLtJ mice	Bcl6 ⁺ IL-17 ⁺ CXCR5 ⁺ CD4 ⁺ T cells (Tfh), Bcl6 ⁺ Foxp3 ⁺ CXCR5 ⁺ CD4 ⁺ T cells (Tfr)	NA	Metformin reduced splenic Tfh cells and increased the splenic Tfr cells in NOD mice. Metformin can ameliorate salivary gland inflammation and hypofunction.
Splenic and Lymphatic Tfh, Tfr ⁸⁸	Lupus-prone mouse (sanroque)	Bcl6 ⁺ CXCR5 ⁺ CD44 ^{hi} CD4 ⁺ T cells (Tfh)↑, Foxp3 ⁺ CXCR5 ⁺ CD4 ⁺ T cells (Tfr)	NA	Tfh cells were increased in salivary gland-draining LN and spleen, but Tfr cells did not change.

Tfh, follicular helper T cells; Tfr, follicular regulatory T cells; Ascl2, achaete-Scute Homologue 2; NA, not applicable; MSCs, mesenchymal stem cells.

further indicates that levels of these cells can be used as a biomarker to evaluate disease activity in patients with pSS. cTfh cells may be a good therapeutic target for these patients.⁶² However, to date, studies linking human cTfh cells to Tfh cells in lymphoid organs and/or ectopic GCs in inflammatory tissues are lacking. There are also no direct functional assays of cTfh cells in vitro and in vivo,^{41,47–53,55,56,60} so we do not know the true function of cTfh cells in helping B cell development in pSS related autoimmunity.

Circulating CXCR5⁺CD4⁺T cells are thought of as a heterogeneous population that contains both Tfh cells and Tfr cells. Tfr cells share a phenotype with Tfh cells. Tfr cells also express surface markers of CXCR5. cTfh cells are defined as CD25⁻CD127^{hi}CXCR5⁺CD4⁺T cells, and they do not increase in patients with pSS. However, CCR7^{lo}PD-1^{hi} subtype of CD25⁻CD127^{hi}CXCR5⁺CD4⁺T cells (cTfh) have been shown to increase in pSS patients with a high degree of focal lymphocytic sialadenitis.⁵⁵ CCR7^{lo}PD-1^{hi}cTfh cells are correlated with dis-

ease activity scores and plasma cells.⁵⁵ CCR7^{lo}PD-1^{hi}Tfh cells can differentiate into mature Tfh cells and induce a GC response upon antigen reexposure.⁴⁰ It suggests that CCR7^{lo}PD-1^{hi}cTfh cells may play an important role in the pathogenesis of pSS disease. When cTfh and Tfr cells are measured simultaneously in other small cases of patients with pSS, there is no significant increase of cTfh cells, activated ICOS⁺PD-1⁺cTfh cells, cTfh1, cTfh2, and cTfh17 cells. The potential effect of any immunosuppressive agents on cTfh and Tfr cells is also excluded.⁶⁰ Therefore, we should carefully exclude Tfr cells in circulating CXCR5⁺CD4⁺T cells, when we assess the frequency and function of cTfh cells in this disease.

As for tTfh cells, CXCR5, CD4, IL-21, and Bcl-6 are important markers, cytokine, and transcription factor respectively (Table 1). IL-21 is found to be at higher levels in the blood of patients with pSS. IL-21 is produced by cTfh cells in patients with pSS⁵¹ and it promotes the B cell proliferation and differentiation as well as aids in the formation of the GC.²⁵ Furthermore, local IL-21 expression is found in the SG, which is expected to increase as more lymphocytes infiltrate into the glandular glands.⁵⁷ IL-21-expressing lymphocytes infiltrating the SGs of patients with pSS also express CXCR5 and are first identified as tTfh cells (IL-21⁺CXCR5⁺T cells) by immunofluorescent staining.⁵⁷ Coexpression of IL-21 and CXCR5 suggests that these tTfh cells are essential for the observed effects of IL-21 in the SGs of patients with pSS. In 2014, salivary CXCR5⁺CD4⁺T cells were reported as tTfh in 24 pSS patients using flow analysis. tTfh cells were positively correlated with blood CD19⁺CD27⁺ memory B cells and CD19⁺CD27^{hi} plasma cells. However, PD-1 and ICOS expression were not measured in these pSS patients.⁴⁹ In another study, ICOS⁺PD-1⁺CD4⁺T cells were thought of as tTfh cells through flow cytometry analysis after enzymatic digestion of the SG from pSS patients. ICOS⁺PD-1⁺CD4⁺tTfh cells were highly expressed in the SG.⁶⁰ We should therefore carefully assess the human salivary tTfh cells due to the loss of CXCR5 during enzymatic digestion.

Maehara et al⁵⁸ indirectly identifies Bcl-6⁺CXCR5⁺T cells (tTfh) in the SG of 54 pSS patients. They show that Bcl-6 mRNA is highly expressed in SGs (SGs) with GC. Tfh-related molecules Bcl-6, CXCR5, and IL-21 are detected in the GC⁺SGs by immunohistochemical staining, and tTfh cells are associated with strong lymphocytic infiltration. However, they do not perform the confocal immunofluorescence staining of tTfh cells related marker in SGs or flow analysis of isolated infiltrated cells from SGs.⁵⁸ Krisztina Szabo shows that Bcl-6⁺CD3⁺T cells (tTfh) are present in the SGs of 10 patients with pSS by confocal immunofluorescence staining. tTfh cell markers (PD-1 and Bcl-6) occur predominantly in more lymphocytic aggregates with higher focus scores in pSS patients. tTfh cells are close to Bcl-6⁺ B cells in ectopic GC, suggesting a crucial role of tTfh cells in promoting GC responses and producing high-affinity antibodies. The presence of tTfh may predict the disease development of pSS.⁵⁹

Besides classic CXCR5⁺Tfh cells, other studies show that CCR9⁺T cells have Tfh-like characteristics and their numbers are both increased in the blood and SGs of patients with pSS.^{63,64} CCR9⁺cTfh cells express PD-1 and ICOS. CCR9⁺cTfh cells have higher expression of IL-7 receptor α and produce high levels of proinflammatory cytokines (IL-21, IFN- γ , IL-17, and IL-4) when triggered with the

antigen or IL-7. Furthermore, CCR9⁺cTfh cells can induce the IgG production of B cells in vitro. CCR9⁺tTfh cells are associated with B cell hyperactivity, autoimmunity, and markers of lymphoid neogenesis.⁶⁴ Enhanced CCL25 (CCR9 ligand) expression in the SGs facilitates the attraction of CCR9⁺cTfh cells into the SGs of patients with pSS, promoting B cell activation, production of autoantibodies, and disease progress.^{64,65}

A recent study using novel epigenetic cell counting methods reveals that an increase in the tTfh cells strongly correlated with CXCL13 expression and B cell hyperactivity in SGs of pSS patients.⁶¹ Abnormality of peripheral tolerance in blood or glandular tissues allows autoreactive mature B cells to undergo extrafollicular or GC responses, which are supported by cTfh and tTfh cells, and differentiate into memory B cells or plasmablasts. This potentially contributes to the development of pSS.

cTfh and tTfh cells are both positively associated with the SGs inflammation, IgG antibody, and disease activity in patients with pSS. Targeting both cell types may prove promising for controlling pSS disease. Investigations of a novel target for Tfh cells in pSS have been reported for many years. Abatacept (CTLA-4Ig) can treat pSS patients by selectively reducing cTfh (CD45RA⁺CXCR5⁺PD-1⁺CD4⁺T) cells in circulation and ICOS⁺CD4⁺T cells in parotid gland tissue. Reduced ICOS expression on cTfh cells is significantly correlated with lower European League Against Rheumatism Sjögren's syndrome disease activity index (ESSDAI) scores during treatment.⁵² Circulating CD19⁺CD27⁺CD38⁺plasmablasts are significantly decreased. Furthermore, serum IL-21 and CXCL13 expression also are decreased. However, glandular IL-21⁺CD4⁺T cells are not affected, maybe due to the short treatment period of only 24 weeks. Interestingly, levels of IgG antibodies to SSA (Ro 52 and Ro 60), and SSB (La) are also decreased, which could be a result of down-regulated Tfh cell-mediated activation of B cells. Increased ICOS expression by Tfh cells provides a stronger secondary costimulatory signal to B cells via ligation of ICOSL.⁶⁶ In turn, this interaction promotes the expression of IL-21 by Tfh cells.⁶⁷ It suggests that ICOS/ICOSL-mediated cross-talk between Tfh cells and B cells is a critical step in pSS-associated B cell hyperactivity.

B cell depletion by Rituximab (RTX) significantly reduces cTfh cells by targeting B cells, leading to improvement in patients with pSS. When RTX therapy is stopped, cTfh cells gradually rise during B cell repopulation.⁵³ There is an indirect effect of RTX therapy in reducing the IL-6 mediated-development of Tfh cells. This work demonstrates potential crosstalk between cTfh cells and B cells through IL-6 signaling in pSS-associated autoimmunity. Calcineurin-inhibitor (e.g., tacrolimus) and the mammalian target of rapamycin (mTOR) inhibitor (e.g., sirolimus) can both inhibit Tfh-like cell differentiation, and reduce the percentage of ICOS⁺Tfh and PD-1⁺Tfh cells from human naïve CD4⁺T in vitro. Furthermore, tacrolimus and sirolimus inhibit human Tfh cell-dependent B cell proliferation and differentiation in vitro. They find the main reason that Tfh cells express less IL-21 in the presence of tacrolimus or sirolimus.⁶⁸ A study shows that methylprednisolone and calcineurin-inhibitors could inhibit the differentiation of human naïve CD4⁺T cells into Tfh cells in vitro.⁶⁹ In another study, treatment with

methylprednisolone decreased the circulating CXCR5⁺PD-1⁺CD4⁺T cells (cTfh), without altering the cTfh function in patients with systemic lupus erythematosus.⁷⁰ It suggests that biologic and immunosuppressive agents may improve the symptoms of human pSS partly through the inhibitory effects of Tfh cells.

There is also some evidence from animal models showing that Tfh cells are involved in the pathogenesis and therapeutic strategy. The biggest advantage of animal models is that many preclinical studies can be rapidly translated from bench to bedside. One study demonstrated that Ascl2, which is reported to induce the differentiation and activation of Tfh cells, is highly expressed in the PBMCs of patients with pSS. Furthermore, overexpression of Ascl2 in NOD mice led to the enhanced expression of CXCR5 in both salivary and lacrimal glands, and expansion of circulating CD4⁺CXCR5⁺, CD4⁺ICOS⁺, and CD4⁺PD-1⁺ cells. These results imply that Ascl2 regulates the differentiation of circulating Tfh cells and tissue Tfh cells in the SS murine model.⁷¹ Murine Tfh17 cells produce IL-17, to promote the differentiation of B cells into GC B cells, which are thought to be the source of pathogenic autoantibodies.⁶² This suggests that Tfh17 cells may be involved in the formation of ectopic GCs in autoimmune diseases. miRNA92a targets KLF2 and phosphatase PTEN signaling to promote Tfh precursors in T1D islet autoimmunity in NOD mice. miRNA92a antagomir can reduce the Tfh precursors in peripheral blood and pancreatic lymph nodes, alleviate immune infiltration, and activation in the pancreas of NOD mice and humanized animal models.⁷² miRNA92a-mediated PTEN signaling may be selected as a target for the treatment of human pSS disease. IL-21 is produced by Tfh and Th17 cells. The pleiotropic IL-21 promotes the differentiation of B cells into plasma cells, expression of Bcl-6, and GC responses. Inhibiting the pathogenic Tfh cells by blocking IL-21 and ICOS reduced the production of autoantibodies in murine lupus and pemphigus vulgaris models.^{73–75} Small-molecule compounds specifically inhibit Tfh cells and attenuate the activity of inflammatory arthritis in the CIA mouse model.⁷⁶ ICOS-PI3K/Akt/mTOR signaling pathway plays a critical role in promoting Tfh cell differentiation. Especially, ICOS can activate mTOR to drive glycolysis and lipogenesis, and glucose transporter 1-mediated glucose metabolism promoting mouse Tfh cell responses.⁷⁷ Therefore, sirolimus can inhibit the function of mouse Tfh cells by suppressing mTOR signaling. Treatment with anti-CD20 antibody inhibited the splenic Tfh cell infusion and autoimmune lesions within the SGs in a mouse model of SS.³⁵ Interesting data showed that treatment with mesenchymal stem cells (MSCs) ameliorates sialadenitis in mice and partly in patients with pSS, by reducing Tfh cells.⁷⁸ These studies suggest a potential therapeutic value of these molecules or cells, targeting Tfh cells for human and animal pSS disease. More data from future studies are waited.

5 | FOLLICULAR REGULATORY T CELLS IN PATIENTS WITH pSS AND ANIMAL MODEL

Tfr cells, described by 3 groups in 2011,^{16–18} are a new subset of CXCR5⁺Bcl-6⁺Foxp3⁺ Treg cells localized in the GC to limit humoral

immune response in humans and mice.^{16–18,79} Like Tfh cells, human and mouse Tfr cells highly express CXCR5; however, they retain the characteristics of Treg cells to express the transcription factor Foxp3 and inhibit Tfh cells and B cells, specifically regulating GC responses and antibody production.^{16–18} Tfr cells are absent in the mouse thymus but can be generated from mouse CXCR5⁺Foxp3⁺ natural Treg cells. Lack of Tfr cells enhances the human GC reaction (higher GL7⁺CD95⁺B cell), affinity maturation of antibodies, and plasma cell differentiation.¹⁴ T cells are stimulated by DCs dependent on transcription factor NFAT2, to up-regulate CXCR5 expression, which promotes T cell migration into the T-B cell junction, to become the precursor of Tfr cells. Later, by stably expressing Bcl-6 and entering the follicular area of B cells, they differentiate into GC Tfr cells in mice.⁸⁰ A recent study demonstrates that the ablation of Bcl-6 in Treg cells promotes the development of characteristic features of Sjögren's syndrome in Bcl6^{fl/fl}Foxp3Cre mice when injected with SG proteins. In contrast, deficiency of Tfh cells prevents such symptoms in Bcl6^{fl/fl}Cd4Cre mice.⁸¹ The above data from human and animal studies show that Tfr cells participate in the regulation of GC responses, Tfh cell function, and affinity maturation of autoantibodies, to essentially manipulate humoral autoimmune diseases like pSS.

The current knowledge on human Tfr cells is limited. Tfr (CXCR5⁺Bcl-6⁺Foxp3⁺) cells have been found within GCs in healthy human tonsils but are much rarer in mice. Due to difficulties in obtaining GC Tfr cells from human lymphoid tissue, circulating Tfr cells (cTfr) have been the focus of investigation in patients with pSS. cTfr cells are found in patients with pSS, but these cells are negative for Bcl-6 expression.^{55,56,60,79,82,83} The main markers of cTfr cells in human pSS are Foxp3, CXCR5, and CD4 (Table 1). Two studies report that the proportion of Tfr cells (Foxp3⁺CXCR5⁺CD4⁺T cells) in blood and the Tfr/Tfh ratio are significantly increased in patients with pSS compared with those of non-SS sicca.^{56,60} One study shows that the cTfr/cTfh ratio is associated with ectopic lymphoid neogenesis, lymphocyte infiltration in the SG of pSS patients. Analysis of the cTfr/cTfh ratio is recommended as a marker for pSS diagnosis.⁶⁰ However, another study does not detect an association between the cTfr/cTfh ratio and inflammation of the gland from pSS patients.⁵⁶ The differences in the results of the above 2 studies may be due to bias in patient selection and their racial composition.

Circulating CD25⁺CD127^{lo}CXCR5⁺CD4⁺T cells (cTfr) are increased in patients with pSS.⁵⁵ cTfr cells can be divided into CXCR3⁺CCR6[−](Th1-like), CXCR3[−]CCR6⁺Th2-like, and CXCR3[−]CCR6⁺(Th17-like). Blood Tfr cells, particularly Th1-like and Th17-like subsets, are significantly correlated with serum IgG levels in pSS patients. Furthermore, Th17-like subsets of Tfr cells are higher in pSS with hypergammaglobulinemia than those without hypergammaglobulinemia.⁵⁵ The increased frequency of cTfr cells in pSS patients is not due to the migration of cTfr cells outside of the inflamed gland because plenty of cTfr cells still exist within ectopic lymphoid structures in the majority of patients. Tfr cells likely result from enhanced differentiation in secondary lymphoid organs due to ongoing humoral responses.⁶⁰ In theory, Tfr cells regulate and limit the GC reaction contributing to the production of antigen-specific

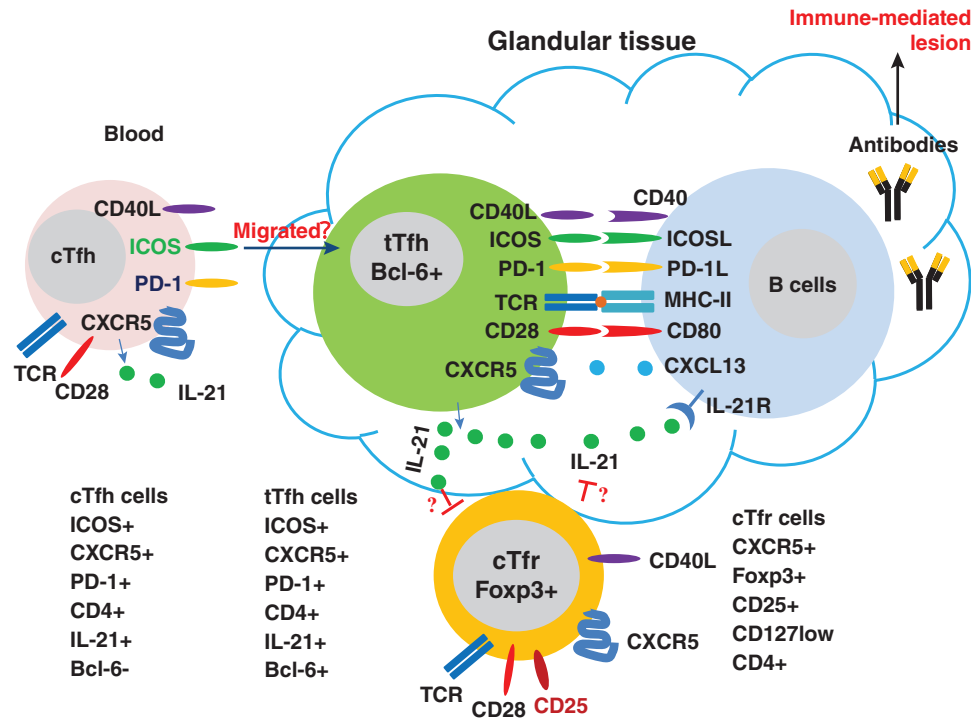


FIGURE 1 Tfh cells and Tfr cells in the immunopathology of primary Sjögren's syndrome (pSS). Tissue Tfh (tTfh) cells and B cells both appear in the glandular tissue of patients with primary Sjögren's syndrome, tTfh cells can help B cell differentiation and formation of antibody-producing plasma via receptor-ligand interaction or secretion of IL-21. Plenty of autoantibodies cause immune-mediated tissue damage in patients with pSS. Circulating Tfh (cTfh) in the blood produce IL-21 and have a similar function like tTfh cells. Circulating Tfr (cTfr) cells may suppress the activity of Tfh cells through against IL-21

antibodies. However, the accumulation of Tfr cells is not sufficient to restore tolerance in exocrine glands, where SS pathogenesis already occurs.⁶⁰ Furthermore, human blood Tfr cells remain immature and are generated before T-B cell interactions are required for the acquisition of follicle access and full regulatory function.⁸⁴ Blood Tfr cells, unlike tissue Tfr cells, have limited suppressive capacity on the humoral response, with no impact on IgG and IgA production. It is reported that cTfr cells are increased after vaccination, blood Tfr cells indicate ongoing humoral responses.^{83,84} Tfr cells inhibit IL-21 and IL-4 expression in Tfh cells in other diseases by suppressing B cell differentiation.²⁵ However, whether these mechanisms of action of Tfr cells on Tfh cells can extend to pSS disease are unknown. Tfr cells are found in the spleen of the animal model of Sjögren's syndrome, but the function of these cells is not mentioned.³⁵ Therefore, peripheral blood expansion of specific Tfr cell subsets suggests ongoing humoral responses and class-switch recombination in human pSS.

Some groups explore the effect of targeting Tfr cells in animal models of Sjögren's syndrome. Recently, the classic drug metformin is shown to suppress CD4⁺T cell differentiation into Th17, Th1, and Tfh cells and enhance that into Treg cells and Bcl6⁺Foxp3⁺CXCR5⁺CD4⁺T cells (Tfr) in vivo and in vitro. Metformin also controls B cell differentiation by reducing GC B (B220⁺GL-7⁺) population and serum IgG levels, reducing SG inflammation, and restoring the salivary flow rate in NOD mice. STAT3, which is downstream of mTOR, is involved in the development of Tfh cells. STAT3-deficient CD4⁺T cells have a defect in Tfh cell differentiation, causing a decrease in GC B cell

development in mice.⁸⁵ These effects of metformin are mediated by the inhibition of AMP-activated protein kinase-dependent mTOR-STAT3 activation. This suggests that metformin is a potential drug partly targeting Tfh and Tfr cells for the treatment of human pSS.⁸⁶ A medicinal herb called Catalpol is shown to reduce lymphocytic infiltration and prevent the formation of ectopic GCs in NOD mice. Although the mechanism of effects of Catalpol in this pSS mice model is unclear. These inhibitory effects are partly dependent on the induction of mouse CD4⁺CXCR5⁺PD-1⁺Foxp3⁺Tfr cells and a higher ratio of Tfr to Tfh cells.⁸⁷

6 | CONCLUDING REMARKS

At present, increasing evidence shows that Tfh cells are increased in the peripheral blood and labial gland tissues of patients with Sjögren's syndrome, and are related to disease activity. Tfr cells with regulatory functions are also found in pSS patients (Fig. 1). However, the nature of Tfh and Tfr cells in SG tissue requires further investigation. The question is whether activated memory Tfh cells, present in the peripheral blood of patients with pSS, originate from inflammatory SG lesions. The information obtained from the single-cell sequencing and immunoproteomics analysis of subsets of cTfh and tTfh cells may answer these questions. Another important question regarding the mechanism by which Tfh and Tfr cells gather at inflammatory lesions and how lymphoid aggregates develop into ectopic GCs can be addressed in mouse

models, but an effort to determine if the observations apply to humans needs to be applied. Currently, there is a lack of specific drugs to inhibit Tfh cells and abate this disease. Improving the old drugs and developing new ones to target Tfh cells would be of great significance.

AUTHORSHIP

W.C., F.Y., G.X., and J.M. wrote the manuscript. W.C. and J.L. reviewed and approved the manuscript.

ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation of China (81701600), Natural Science Foundation of Zhejiang Province (LQ17H100001 and LGF18H100001), and the Research Medical and Health Program of Zhejiang Province (2019RC032).

DISCLOSURES

The authors declare no conflicts of interest.

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How to cite this article: Chen W, Yang F, Xu G, Ma J, Lin J. Follicular helper T cells and follicular regulatory T cells in the Immunopathology of Primary Sjögren's Syndrome. *J Leukoc Biol*. 2021;109:437-447. <https://doi.org/10.1002/JLB.5MR1020-057RR>